Bioequivalence Study Design, Regulatory Requirements and Challenges for Generic Drug Approval: A Global Perspective

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***Abstract*—Bioequivalence studies provide the scientific and regulatory basis for approval of generic drug products all over the world as they allow proof of therapeutic equivalence to the innovator reference product, thereby avoiding the need for complete clinical efficacy testing. The present review covers comprehensively the various principles for the design of BE studies, including the pharmacokinetic parameters, BCS classification, the crossover and parallel design, the fasting-state and fed-state, the statistical methodology, as well as subject selection criteria. Comparative assessment of the regulatory requirements of the major regulators USFDA, EMA, WHO, CDSCO, Health Canada and PMDA indicates that there are many similarities in the fundamental concepts, and differences in the expectations for highly variable drugs, narrow therapeutic index drugs, biowaiver eligibility and bioanalytical method validation. There is a critical discussion on special considerations for complex drug categories, modified-release formulations, endogenous compounds and ethnically diverse populations. The ICH M10 guideline has provided an appreciable harmonization to the bioanalytical method validation requirements and the ICH M13A project has great potential for more harmonization of BE study requirements worldwide. New technologies such as physiologically-based pharmacokinetic (PBPK) modeling, in vitro-in vivo correlation, adaptive study designs, and artificial intelligence-based prediction models are explored for their potential to improve generic drug development and alleviate clinical study burden. Today, the need for international cooperation and science-based regulatory development is highlighted by persistent challenges such as regulatory heterogeneity, resource limitations in developing countries and the complexity of highly variable and locally acting drug products.**

***Index Terms*—Bioequivalence, Pharmacokinetics, BCS Classification, Highly Variable Drugs, Regulatory Harmonization, Generic Drug Approval**

I. Introduction

The last 30 years have seen the generic drug market grow to unprecedented levels in the global pharmaceutical industry, largely due to the expiration of patents and exclusivity periods of many blockbuster drug products [1]. In both developed and developing countries, generic medicines are an essential part of making health care affordable and effective, and an important part of public health sustainability. The successful approval of drug products in the generic category is based upon the demonstration of therapeutic equivalence with the innovator reference listed drug (RLD), with bioequivalence (BE) studies as the basis of approval [2]. The regulatory agencies around the world, such as the Food and Drug Administration (USFDA), the European Medicines Agency (EMA), World Health Organization (WHO), and Central Drugs Standard Control Organisation (CDSCO) of India, have developed extensive guidelines to regulate the design, conduct, and assessment of BE studies, with a core aim of ensuring patient safety while also permitting access to more affordable generic alternatives [3].

Bioequivalence is generally defined as the similarity of two pharmaceutical products with respect to the rate and/or extent to which the API reaches the site of drug action when the products are administered under similar conditions at the same molar dose in an appropriate study [4]. The principles behind BE assessment are based on the concept of bioavailability (BA), which is the proportion of administered dose which reaches systemic circulation unchanged and rate at which this occurs. BA and BE have emerged as scientifically valid and regulatory accepted paradigms that can be used to establish therapeutic equivalence in lieu of the potentially expensive and ethically questionable clinical efficacy trials required for all generic products [5]. Although BE science and regulatory science have advanced over the last decades, there are still a lot of differences between international regulatory guidelines in terms of study design, acceptance criteria, requirements for subject selection, bioanalytical validation requirements, and the handling of special drug categories, including highly variable drugs (HVDs), narrow therapeutic index (NTI) drugs, and complex dosage forms [6]. This non-harmonisation creates significant scientific, financial and logistic burden for pharmaceutical manufacturers, especially those in developing economies that would like to have access to several markets at one go. In this context, there has been a strong push by international regulatory collaboration groups, in particular the Bioequivalence Working Group for Generics (BEWGG) of the International Pharmaceutical Regulators Programme (IPRP) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), to converge regulations to meet this challenge, most notably the ICH M13A guideline on BE for immediate-release solid oral dosage forms [7].

The present review covers in detail scientific aspects of BE study design, critically reviews the regulatory requirements governing BE studies in major jurisdictions, discusses special considerations for complex drug categories, reviews bioanalytical method requirements, discusses some of the key challenges facing the BE field, and covers emerging technologies and future directions that are expected to shape the BE assessment landscape globally.

II. Fundamentals of Bioequivalence

2.1 Definition and Concept of Bioequivalence

The concept of bioequivalence is based on the principle of pharmacokinetics that two drug products that contain the same API in equivalent doses will have similar therapeutic effects only when they expose the body to the same amount of API [8]. Pharmaceutical equivalence means that drug products contain the same amount of the same active substance, the same dosage form and the same or comparable standards, bioequivalence is the demonstration of pharmacological equivalence in the human body [9]. It is important to note that just because a drug is Pharmaceutical Equivalent does not necessarily mean it is Bioequivalent: Small differences in excipients in the formulation, small differences in particle size distribution, small differences in polymorphic form of the API, small differences in the manufacturing process and small differences in dissolution characteristics can have a profound impact on the rate and extent of drug absorption. Thus, in vivo BE studies are the gold standard and most widely accepted regulatory approach to determine therapeutic equivalence [10]. Therapeutic equivalence, the ultimate goal of generic drug approval is assumed when two pharmaceutical products are pharmaceutically equivalent and bioequivalent. This assumption underpins the science and regulation that underlies global generic substitution policies, which allow clinicians and pharmacists to interchange generic products with brand name innovator products without concerns for clinical interchangeability [11].

2.2 Pharmacokinetic Parameters in BE Studies

The quantitative assessment of BE is mainly based on the pharmacokinetic (PK) parameters obtained from the plasma concentration-time curves after administration of test and reference products. The total systemic exposure (AUC) is regarded as the most important parameter for evaluation of the degree of drug absorption [12]. AUC is usually measured from time zero to the last measurable concentration (AUC_{0-t}) and to infinity ($AUC_{0-\infty}$) using the terminal elimination rate constant. Maximum systemic exposure, represented as peak plasma concentration (C_{max}), is a reflection of the rate of absorption and indirectly of the safety profile of the drug product, especially in case of adverse effects related to plasma concentration. The time to peak concentration (T_{max}) is a measure of the rate of absorption, and is not routinely assessed for BE as it is a discrete value. Partial AUC can be used for the modified-release formulations and other parameters like $AUC_{t_1-t_2}$ can be used to evaluate the release or absorption in more detail [13].

2.3 BCS Classification and Its Role in BE

In 1995, Amidon and colleagues developed the Biopharmaceutics Classification System (BCS) which creates a scientific classification of drug substances based on two key determinants of oral drug absorption – aqueous solubility and intestinal membrane permeability. BCS Class I drugs are highly soluble and highly permeable, hence they are rapidly and completely absorbed, whereas BCS Class II drugs are poorly soluble but highly permeable, with dissolution being the rate limiting step in absorption [14]. BCS Class III drugs are characterised by high solubility and low permeability, where the bioavailability is controlled by the membrane transport system, while BE assessment is more complex in BCS Class IV drugs, due to dual issues of low solubility and low permeability thus affecting their formulation [15]. The BCS framework has been widely embraced by regulatory agencies around the world for providing regulatory exemption from in vivo BE studies for some drug products to facilitate generic drug approval, which also saves costs and ethical concerns of in vivo BE studies [16].

2.4 Types of BE Studies

There are several approaches to assess bioequivalence. The most common and required approach for most drug products is in vivo pharmacokinetic studies, which include the measurement of drug concentration in plasma, serum or urine [17]. Pharmacodynamic (PD) BE studies, which look at the pharmacodynamic activity of the test product compared to the reference product, are used when plasma drug concentrations are not reliably measurable, such as for some topically acting drugs and corticosteroids [18]. Clinical endpoint BE studies are used to show therapeutic equivalence and are reserved for complex drug products where neither PK or PD endpoints are possible (such as nasal sprays, orally inhaled products and topical dermatological preparations) [19]. In vitro BE approaches, such as comparative dissolution testing and the BCS-based biowaiver methodology are non-clinical alternatives that can be applied under certain regulatory and scientific circumstances. As mechanistic tools to support or supplement the traditional in vivo BE assessments, in silico approaches such as physiologically based pharmacokinetic (PBPK) modeling and in vitro-in vivo correlation (IVIVC) are gaining interest [20].

III. Bioequivalence Study Design

3.1 Crossover vs Parallel Study Design

The key principle of BE study design is to reduce the difference in experimental variability and to increase the statistical power to detect a meaningful difference in drug exposure between the test product and the reference product [21]. It is generally accepted that the randomized, two-period, two-sequence, two-treatment crossover design is the most statistically efficient and preferred design for BE studies since each subject is serving as his or her own control, so that inter-subject variability is not a statistical concern

when comparing the treatment effects. In this design, each subject is randomly assigned to one of two sequences of treatment, test/reference or reference/test, and there should be a sufficient washout period between the test and reference treatments to allow complete washout of the drug from the first treatment period [22]. The parallel-group design, where the subjects are randomly assigned to one treatment (test or reference product) and each subject is only measured once (at one treatment period), is usually less statistically efficient because it includes between-subject variability as a component of the error term. For drugs with very long elimination half lives, however, where adequate washout would be practically impossible, for drugs with irreversible pharmacological effects and/or for special population studies (e.g. oncology patients, pediatric subjects) where repeated dosing is ethically problematic, however, parallel designs are necessary and scientifically appropriate [23].

3.2 Fasting vs Fed State Studies

Physiological status of gastrointestinal tract at the time of drug administration has significant influence on drug dissolution, gastrointestinal transit and absorption. Mechanisms by which food changes bioavailability of orally administered drug products include modification of gastric pH, increase in splanchnic blood flow, changes in bile secretion, delay in gastric emptying and changes in the viscosity of food in the lumen [24]. Regulatory guidance from the USFDA and most International authorities suggests that an oral solid dosage form should, at the minimum, be assessed using a primary fasting BE study, to avoid the confounding effect of food on the drug absorption and to maximize the discriminating nature of the study for the purpose of detecting difference between formulations [25]. A fed BE study is also needed if the product is labeled for use with food, if there is known food-drug interaction, or if there is a possibility of food-drug interaction based on the physicochemical properties of the drug (e.g., highly lipophilic drugs or pH dependent drugs). The requirement for fed studies is done on a case-by-case basis, as determined by the EMA and other regulatory agencies; it is based on the conditions outlined in the prescribing information of the comparator product, thus it is a risk-based and individualized approach to regulation [26].

3.3 Sample Size and Power Calculations

The sample size of a BE study should be planned in advance using a robust statistical power calculation, where the variance of the test and reference product pharmacokinetic parameters under evaluation, the within-subject variability, the level of statistical significance, the statistical power (usually 80% or greater), and the predicted geometric mean ratio between the test and reference product are all considered. The most important input for sample size estimation is the within-subject coefficient of variance (CV%) from published literature, previous studies or pilot investigations [27]. Guidelines for regulatory authorities globally state that no BE study should have fewer than 12 evaluable subjects. As observed by multiple regulatory authorities including the USFDA, the EMA, Health Canada and WHO, adaptive and sequential study designs like the group sequential design are also allowed, when the within-subject variability for a given drug is unknown or uncertain, the first stage of the study gives an estimate of the within-subject variability, which is then used to calculate the number of the additional subjects needed to obtain the desired power [28].

3.4 Subject Selection Criteria

BE studies are traditionally performed in healthy adult volunteers aged 18 to 55 years because this age group is likely to have the least number of factors that could contribute to variability of drug pharmacokinetics and is therefore the best population in which to compare the effects of drug formulations [29]. Commonly, subjects must be within normal body mass index, be a non-smoker or have minimal smoking, have no clinically significant medical conditions, and not be taking any other medication that may

affect drug absorption, distribution, metabolism or excretion [30]. This issue of sex distribution of study subjects has been one that has been evolving through regulatory guidance, but now most regulatory bodies recommend the use of both males and females in BE studies, as the study population should reflect the population of patients for which the drug is intended. Specific genetic polymorphisms of drug metabolizing enzymes, such as poor metabolizers for CYP2D6 or ultra-rapid metabolizers may warrant special attention for drugs with polymorphic metabolism, and genotyping of subjects is increasingly recommended for pharmacogenomically relevant drug compounds. Because Japanese population has a greater prevalence of achlorhydria than western population, Japanese attention is focused on subjects with low gastric acidity for drugs with pH-dependent solubility [31].

3.5 Reference and Test Product Selection

An important regulatory criterion which affects the validity and international acceptability of the results from BE studies is the choice of the reference product. The reference product is typically the first marketed drug product in the country of regulatory submission, and there is a need to justify the choice of reference product in terms of availability of long term clinical safety and efficacy data. The USFDA identifies the specific reference listed drugs (RLDs) for each ANDA submission in the Orange Book and EMA defines the reference medicinal product as the innovator product approved in any EU member state [32]. As the innovator product is not always locally available, WHO guidelines permit the use of a comparator product, which is recognized internationally, particularly in the case of generic manufacturers in developing countries. The batch of the test product used in the pivotal BE study should be representative of the planned commercial production batch in terms of composition, manufacturing process, batch size, dissolution characteristics, and be consistent with current Good Manufacturing Practice (cGMP) requirements [33].

3.6 Statistical Analysis 90% Confidence Interval Approach

Two one-sided tests (TOST) procedure is the statistical approach universally followed for BE evaluation and is mathematically equivalent to the construction of a 90% confidence interval (CI) for the ratio of the geometric means of the pharmacokinetic parameters of interest. BE is concluded when the 90% CI for the test/reference geometric mean ratio of AUC and Cmax is within the predefined acceptance range of 80.00% to 125.00% for both the AUC and Cmax. These acceptance limits are in logarithmic units; they indicate the regulatory acceptability of a 20% or less difference in drug exposure between the test product and the reference product for the vast majority of drug substances [34]. Log-transformation of PK data before statistical analysis is required by all major regulatory authorities, given that it is the correct transformation to account for the multiplicative nature of the PK variability, and to meet the distributional assumptions of the parametric statistical models used. The common statistical model used for crossover BE studies is analysis of variance (ANOVA) with appropriate model terms for sequence, period, subject nested within sequence, and treatment [35].

Table 1: Comparison of Bioequivalence Study Design Parameters Across Major Regulatory Jurisdictions [36–38]

Parameter	USFDA	EMA	WHO	CDSCO	Health Canada	PMDA (Japan)
Key Regulatory Document	21 CFR Part 320; FDA Guidance (2021)	CHMP Guideline on BE (2010)	WHO TRS 1003, Annex 6 (2022)	New Drugs & Clinical Trials	Health Canada Guidance (2018)	MHLW/PMDA Guidance

				Rules, 2019		
Preferred Study Design	Single-dose, 2-period, 2-sequence crossover	Single-dose, 2-period, 2-sequence crossover	Single-dose, 2-period, 2-sequence crossover	Single-dose, 2-period, 2-sequence crossover	Single-dose, 2-period, 2-sequence crossover	Single-dose, 2-period, 2-sequence crossover
Minimum Subjects	12 evaluable	12 evaluable	12 evaluable	12 evaluable	12 evaluable	12 evaluable
Fasting Study	Mandatory for IR forms	Mandatory	Mandatory	Mandatory	Mandatory	Mandatory
Fed Study	Required if labeled with food or food effect expected	Case-by-case per SmPC	Required if food effect anticipated	Required if labeled with food	Required if food effect anticipated	Required if label specifies food
AUC Acceptance Criteria	90% CI: 80–125%	90% CI: 80–125%	90% CI: 80–125%	90% CI: 80–125%	90% CI: 80–125%	90% CI: 80–125%
Cmax Acceptance Criteria	80–125%; RSABE for HVDs	80–125%; scaled approach for HVDs	80–125%	80–125%	80–125%; scaled acceptable	80–125%
HVD Approach	RSABE; replicate design; sWR-based scaling	Scaled ABE; k=0.760; GMR within 80–125%	Increased sample size recommended	Increased sample size	Reference-scaled for Cmax	Replicate design; case-by-case
NTI Drug Criteria	Tightened; AUC 90–111.11%	90% CI: 90–111.11% for AUC	Tightened criteria	Tightened; case-by-case	90% CI: 90–112% for AUC	Tightened; case-by-case
Primary PK Parameters	AUC_{0-t}, $AUC_{0-\infty}$, Cmax	AUC_{0-t}, $AUC_{0-\infty}$, Cmax	AUC_{0-t}, $AUC_{0-\infty}$, Cmax	AUC_{0-t}, $AUC_{0-\infty}$, Cmax	AUC_{0-t}, $AUC_{0-\infty}$, Cmax	AUC_{0-t}, $AUC_{0-\infty}$, Cmax
Preferred Analyte	Parent compound; metabolite if needed	Parent compound preferred	Parent compound preferred	Parent compound preferred	Parent compound preferred	Parent compound; metabolite in

						specific cases
Adaptive Design	Permitted; two-stage with pre-specification	Permitted; two-stage with alpha-spending	Permitted with pre-specification	Not explicitly defined	Permitted with pre-specified plan	Permitted with regulatory pre-approval
Bioanalytical Standard	ICH M10 (2022); FDA BMV Guidance	ICH M10 (2022)	ICH M10 (2022)	ICH M10 (2022)	ICH M10 (2022)	ICH M10 (2022)
GCP Requirement	ICH E6; IRB approval	ICH E6; Ethics Committee approval	ICH E6; Ethics Committee approval	ICH E6; IEC per ICMR guidelines	ICH E6; REB approval	ICH E6; IRB approval
Reference Product	RLD per FDA Orange Book	EU-authorized reference medicinal product	WHO-recognized comparator product	CDSCO-approved innovator product	Canadian Reference Product	MHLW-approved comparator product

IV. Global Regulatory Frameworks for BE Studies

4.1 US FDA Guidelines and Requirements

The USFDA has the most scientifically detailed and documented regulatory framework for assessment of BE studies worldwide, which is mainly regulated by the Code of Federal Regulations (CFR) Title 21, Part 320 and is supplemented by a comprehensive set of guidance documents for industry published by the Center for Drug Evaluation and Research (CDER). In the U.S., applications to approve generic drugs are filed as Abbreviated New Drug Applications (ANDAs) under Section 505(j) of the Federal Food, Drug and Cosmetic Act and must show pharmaceutical equivalence and BE to a designated RLD [39]. The FDA guidance documents span a broad range of topics that are relevant to BE and include general guidance documents for BE studies submitted in NDAs or INDs, product-specific guidance (PSG) documents that provide detailed guidance for BE studies for individual generic drug products, guidance on the conduct of bioanalytical method validation, and guidance for complex drug products, modified release, locally acting or complex mixtures. The FDA uses the average bioequivalence (ABE) approach with the 90% CI acceptance criterion of 80.00 – 125.00% for AUC and Cmax as the default framework, but has specific statistical approaches for highly variable drugs (e.g., the reference-scaled average bioequivalence [RSABE] approach) and for narrow therapeutic index drugs (e.g., the individual bioequivalence [IBE] approach, which uses tightened acceptance criteria and scaled approaches to reflect the drug's unique therapeutic risk profile) [40].

4.2 European Medicines Agency (EMA) Guidelines

The Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) regulates BE studies via the Guideline for the Investigation of Bioequivalence (BIQ) which represents a comprehensive regulatory document of all the BE related submissions in European Union [41]. The EMA guideline is very similar to the USFDA in many basic principles of BE Design and Statistical Analysis, including the use of the 90% CI and limits 80.00 – 125.00% for AUC and Cmax. But there are some key distinctions between the EMA's method in specialized fields. For drugs that exhibit high variability, the EMA allows an alternative approach for Cmax, where the acceptance interval can be larger than the 80.00–125.00% range, using the reference product within-subject standard deviation (sWR) in the algorithm $[L, U] = \exp[\pm k \times sWR]$ and a regulatory constant of $k = 0.760$, with 80.00–125.00% for GMRA for AUC remaining. Comparative dissolution study for different pH's is also highlighted in the EMA guideline as part of the BE dossier and detailed instructions are given for the food effect and handling of metabolites and enantioselective analysis on a product specific basis [42].

4.3 World Health Organization (WHO) Guidelines

The World Health Organization (WHO) has a central role in harmonization of BE regulatory requirements between the developing and middle-income countries through its Technical Report Series on multisource pharmaceutical products (MPMP), which offers internationally accepted guidance for the design, conduct and evaluation of BE studies of generic medicines. WHO guidance has special relevance for countries without developed technical and regulatory capacity for creating fully independent BE frameworks, and many national regulatory authorities in Asia, Africa and Latin America have used WHO guidance directly or as a key reference point for their own guidance [43]. With regard to study design preference, statistical analysis methodology and acceptance criteria, the WHO framework is broadly in line with international standards, but has been rather progressive in extending the range of biowaivers that can be used for BCS-based exemptions. Of particular importance is the WHO's policy of approving BCS-based biowaivers for Class III drugs, which is more liberal than the USFDA's stance of not routinely granting biowaivers for Class III compounds, reflecting the support of the WHO for efforts to lower economic obstacles for access to generic drugs in resource-poor regions [44].

4.4 Indian Regulatory Framework CDSCO

The Central Drugs Standard Control Organisation (CDSCO), under the Ministry of Health and Family Welfare (MHFW), India, regulates BA and BE studies under the New Drugs and Clinical Trials Rules, 2019, and related guidance documents. India is one of the world's largest and most prolific generic drug manufacturing hubs, and Indian pharmaceutical companies are among the world's leading suppliers of generic medicines to regulated markets such as the United States, the United Kingdom and the European Union. The requirements for study design, choice of subjects, validation of analytical method, data reporting and review by ethics committee in the guidelines for BA/BE studies are broadly similar to the international standards. In vitro dissolution studies and BA/BE studies in India must conform with the requirement of schedule Y of the Drugs and Cosmetics Act and clinical BE studies must be carried out in clinical pharmacology units having proper facilities of bioanalytical labs. The CDSCO has been making constant efforts to modernize its regulatory guidelines and bring them closer to ICH and WHO standards, thereby signaling India's intention to move towards increased regulatory harmonization and mutual recognition of BE data in various global markets [45].

4.5 ICH Guidelines Relevant to BE

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has played a significant role in the global BE regulatory harmonization efforts by issuing a

number of important guidelines. ICH E6 (Good Clinical Practice) provides universal standards for the ethical and scientific conduct of clinical studies, including BE studies, including requirement for investigator qualifications, informed consent, data integrity and monitoring of the studies. ICH M10 on Bioanalytical Method Validation, completed in 2022, is a milestone in global harmonization in bioanalytical standards and provides harmonized requirements for the validation of chromatographic and ligand-binding assay methods for the quantitative determination of drugs and metabolites in biological matrices. Most importantly, the ICH M13A guideline on BE for immediate-release solid oral dosage forms, which has reached Step 2 endorsement in December 2022, is expected to harmonize the main requirements for BE studies for all the ICH member regions and thus may be the most impactful step toward global BE regulatory harmonization in decades [46].

Table 2: Comparative Overview of Global Regulatory Guidelines for Bioequivalence Studies [47–49]

Parameter	USFDA	EMA	WHO	CDSCO	Health Canada	PMDA (Japan)
BCS Class I Biowaiver	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted
BCS Class II Biowaiver	Not routinely; limited cases for weak acids	Not routinely accepted	Conditionally for some acids	Not routinely accepted	Not routinely accepted	Not routinely accepted
BCS Class III Biowaiver	Not routinely accepted	Accepted if very rapidly dissolving; Q1/Q2 same	Accepted; most permissive globally	WHO guidance referenced	Accepted under specific conditions	Not routinely accepted
BCS Class IV Biowaiver	Not accepted	Not accepted	Conditionally in specific cases	Not accepted	Not accepted	Not accepted
Rapid Dissolution Criterion	≥85% in 30 min	≥85% in 30 min (very rapid: 15 min)	≥85% in 30 min	≥85% in 30 min	≥85% in 30 min	≥85% in 30 min
Dissolution Media (pH)	1.2, 4.5, 6.8	1.2, 4.5, 6.8	1.2, 4.5, 6.8	1.2, 4.5, 6.8	1.2, 4.5, 6.8	1.2, 4.5, 6.8
Profile Similarity Criterion	f2 ≥ 50	f2 ≥ 50	f2 ≥ 50	f2 ≥ 50	f2 ≥ 50	f2 ≥ 50
Solubility Criterion	Highest dose in	Highest dose in	Highest dose in 250	Highest dose in	Highest dose in	Highest dose in 250

	250 mL; pH 1–7.5; 37°C	250 mL; pH 1–6.8; 37°C	mL; pH 1.2–6.8; 37°C	250 mL; pH 1–7.5	250 mL; pH 1–7.5	mL; physiological pH
Permeability Criterion	≥90% absorbed; human data preferred	≥85% absorbed; in vitro with justification	≥85% absorbed; human data preferred	Human or validated in vitro data	≥85% absorbed; human or in vitro	Human data preferred; in vitro acceptable
Excipient Restrictions	Must not affect absorption; surfactants flagged	Q1/Q2 sameness for Class III; no absorption effect	Flagged excipient list provided	Must not affect absorption	Must not alter absorption	Must not influence absorption
NTI Drugs Eligible	No	No	No	No	No	No
Strength Waivers	Permitted; highest strength BE + proportional composition	Permitted; proportional composition + dissolution	Permitted with dissolution data	Permitted with dissolution data	Permitted with dissolution data	Permitted; dissolution profile comparison
Eligible Dosage Forms	IR solid oral dosage forms only	IR solid oral dosage forms only	IR solid oral dosage forms; oral solutions separately	IR solid oral dosage forms	IR solid oral dosage forms	IR solid oral dosage forms

V. Biowaivers and BCS-Based BE Exemptions

5.1 Concept and Scientific Rationale for Biowaivers

A biowaiver is a scientific and regulatory pathway that allows for the waiver of an in vivo BE study for a generic drug product provided that in vitro pharmaceutical equivalence can be demonstrated. The biowaiver concept is based on the well-defined knowledge that certain drugs with specific kinetic properties, especially those where the absorption is not dissolution rate limited, can be predicted by dissolution testing in vivo. Biowaivers can thus only be considered if there is strong and predictable correlation between in vitro dissolution and in vivo absorption, which is most well-established for highly soluble and highly permeable drug substances. A biowaiver will save pharmaceutical manufacturers time, cost, and resources, and minimize the ethics concern of using human subjects for clinical pharmacokinetic testing. Biowaivers also help to more efficiently use review resources for regulatory authorities that have an ever-growing workload from generic drug applications [50].

5.2 BCS Class I and Class III Biowaivers

BCS Class I drugs have high aqueous solubility and high intestinal permeability, and they are the easiest to consider for biowaiver approval because absorption is more likely to be absorption-limited than dissolution-limited. For BCS Class I drug products, which are also found to have rapid dissolution defined by FDA as ≥ 85% dissolution within 30 minutes in pH 1.2, 4.5 and 6.8 dissolution media, the in vitro dissolution data alone is sufficient to predict in vivo BE provided that excipients used does not have significant effect on the rate or extent of absorption. The scientific basis of Class I biowaivers is well established and accepted by all major regulatory authorities such as USFDA, CDSCO, EMA and WHO. BCS Class III drugs, with high solubility and low permeability, are a more complex situation in terms of biowaiver eligibility. The EMA acknowledges that for very rapidly dissolving products, the absorption is not driven by dissolution but by membrane permeability, and allows for BCS Class III biowaivers when the excipients composition of the test and reference products are very similar qualitatively and quantitatively. WHO has relaxed its biowaiver requirement to cover all BCS classes under appropriate conditions, and is the most liberal regulatory authority in terms of biowaiver globally, whereas the USFDA currently takes a more conservative stance towards Class III biowaivers [51].

5.3 Regulatory Acceptance and Comparative Dissolution Requirements

All biowaiver applications irrespective of BCS class should be submitted with comparative dissolution profiles from the test product and the reference product performed at a minimum of three different pH levels (1.2, 4.5 and 6.8) to cover the gastrointestinal physiological pH range. Dissolution profiles are compared for similarity by calculating the similarity factor f2, which is conventionally considered a similarity if the f2 value is > 50. If rapid dissolution (≥85% in 15 minutes) is proven for both test and reference products at all three pH, then the profile comparison with the f2 metric may not be necessary because both products are expected to be completely dissolved before arriving at the site of intestinal absorption. One important factor is the quality of excipients used in the test formulation, as some excipients such as the surfactants, cyclodextrins, and some polymers are known to affect drug absorption due to their effect on membrane permeability, efflux transporter inhibition or gastrointestinal motility, and therefore might compromise the biowaiver rationale [52].

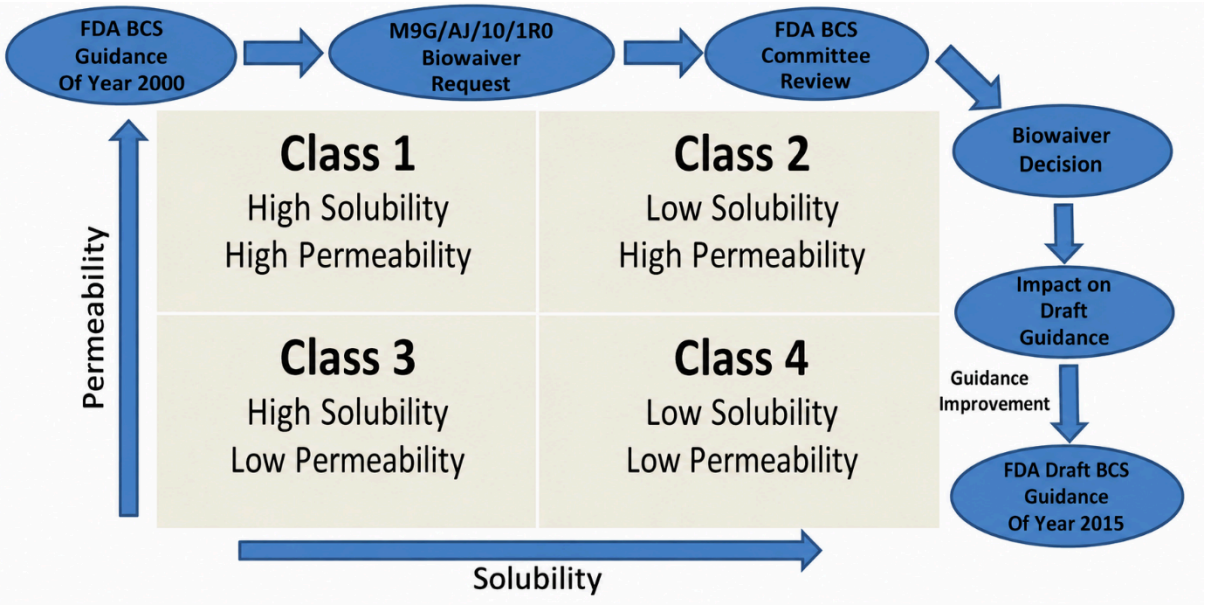

Figure 1: BCS Classification System and Biowaiver Eligibility

VI. Special Considerations in BE Study Design

6.1 Highly Variable Drugs and Reference-Scaled Average BE

Highly variable drugs (HVDs) are those drugs that have a within-subject coefficient of variation (CV%) ≥ 30% for one or more major pharmacokinetic (PK) parameters, especially for C_{max}. This is a fundamental statistical issue of conventional BE studies because high intra-subject variability is inherent in HVDs, and sometimes requires unfeasibly large numbers of subjects (n>100) to conclude BE within the standard 80-125% acceptance limits, which is economically and ethically impractical. In response to this scientific and regulatory issue, the USFDA has proposed the reference-scaled average bioequivalence (RSABE) approach, which scales the BE acceptance criterion for C_{max} based on the within-subject variability for the reference product, and thus broadens the BE acceptance interval for drugs with high within-subject variability. The parallel scaled average BE approach is used for C_{max} and the acceptance limits are calculated as a function of the sWR with a regulatory constant k = 0.760, with the further requirement that the geometric mean ratio must not exceed the 80.00–125.00% range. These approaches are scientifically valid because the extent of the variability is an inherent characteristic of the drug substance and not something that is only caused by the formulation, thus the demonstration of the equivalence of the formulation should not be penalized for variability that also occurs with the reference product [53].

6.2 Narrow Therapeutic Index Drugs

At the other end of the spectrum to HVDs are narrow therapeutic index (NTI) drugs, which include warfarin (anticoagulants), cyclosporine and tacrolimus (immunosuppressants), phenytoin and carbamazepine (antiepileptics), and digoxin (cardiac glycosides). Even differences in systemic exposure of small magnitude may lead to clinically relevant outcomes for these drugs, including lack of efficacy, serious side effects, and toxicity . Thus, the BE acceptance criteria for NTI drugs are tighter or stricter in most of the regulatory authorities. For some NTI drugs, the USFDA has specified that individual bioequivalence test shall be performed or a scaled approach with tighter regulatory constants shall be adopted, whereas the EMA has specified the acceptance range for the 90.00–111.11% CI for the geometric mean ratio for AUC. The Canadian regulatory agency, Health Canada, also has stricter acceptance criteria for NTI drugs, and there is universal agreement among global regulators that the benefit-risk assessment for an NTI drug substitution requires a higher level of established pharmacokinetic equivalence than do drugs with broader therapeutic windows [54].

6.3 Modified Release Formulations

Oral dosage forms with modified-release properties (MR) include extended-release, delayed-release, and pulsatile-release products, and their drug release characteristics are specially designed to be fundamentally different from the characteristics of immediate release products, which poses unique and multifaceted challenges for BE study design. Along with equivalent total drug exposure and peak concentration, BE studies of MR products should establish that the extended or controlled release properties of the test product are reproduced to the same degree as the reference product, thus reproducing the desired pharmacokinetic profile and the therapeutic, tolerability, and patient compliance benefits associated with it. Fasting and fed BE studies are usually needed for MR products, because the effects of food on the release of drug from extended-release matrix systems can be quite large and clinically significant, especially for drugs that exhibit dose dumping phenomena when fed with alcohol or high fat meals. Partial AUC measurements at well-defined time points may be needed to describe the early, middle and late components of the drug absorption from MR formulations and the EMA makes clear guidance on the choice of the partial AUC cutpoints depends on the pharmacokinetic profile of the reference product [54,55].

6.4 Complex Drug Products

The scientific frontier of BE assessment is represented by complex drug products such as topical dermatological preparations, transdermal delivery systems, orally inhaled products (OIPs), nasal drug products, ophthalmic suspensions and liposomal formulations. Plasma concentration-based pharmacokinetic studies may not be sufficient for demonstrating clinically relevant differences in formulation for locally acting topical drug products that act primarily at the local site of action (skin or mucosa). The USFDA has provided product-specific guidance for individual complex generic drug products that describe specific BE approaches including product sameness testing (qualitative and quantitative compositional equivalence, or Q1/Q2 testing), pharmacodynamic endpoint testing (using validated models, e.g., vasoconstrictor assay with corticosteroids), and in vitro permeation testing (IVPT) using human skin. The determination of aerodynamic particle size distribution (APSD) by cascade impaction and proof of equivalence in fine particle mass (FPM) delivered to the lungs are important BE endpoints for products that are delivered via the oral inhalation route and can be used to supplement or replace classical PK studies [56].

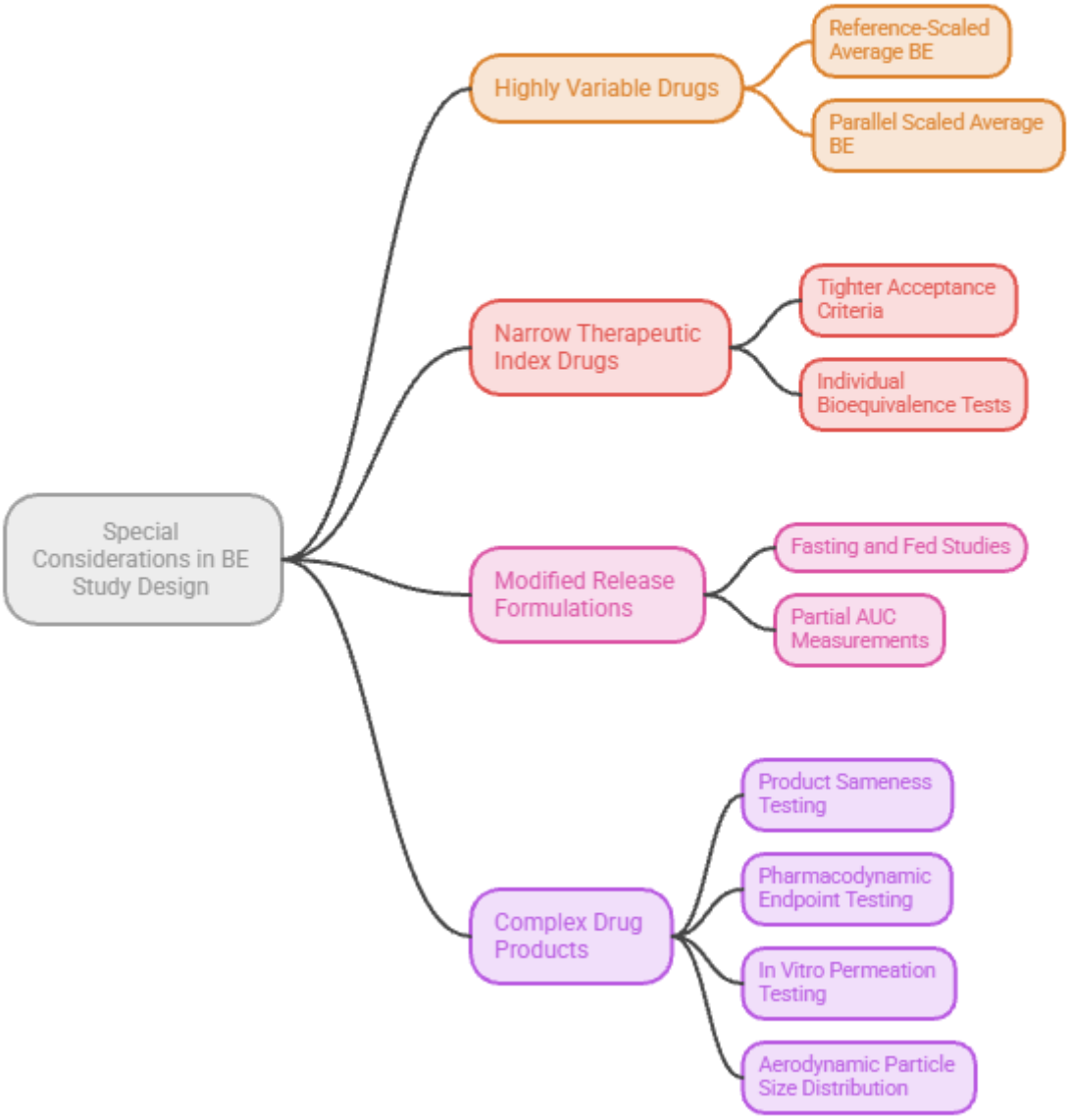

Figure 2: Considerations for selection of bioequivalence study design

VII. Analytical Methods in BE Studies

7.1 Bioanalytical Method Development and Validation

The reliability and integrity of bioanalytical data obtained during BE studies is key to the scientific validity and regulatory acceptance of BE conclusions. Before they can be used on study samples, the bioanalytical methods must be fully validated to assure that they are specific, sensitive, accurate, precise, linear and robust for the range of concentrations expected in subject samples. When developing a suitable bioanalytical method, careful evaluation of the physicochemical properties of the analyte, as well as the identification and resolution of potential interferences from the endogenous matrix components, metabolites, and/or co-administered substances, and optimization of the sample preparation procedures to ensure adequate recovery of the analyte and minimal matrix effects are among the key considerations. Selectivity, the ability of the method to measure the analyte of interest without interference from other substances present in the biological matrix is of special importance for drugs that are extensively metabolised in the first pass or administered as a prodrug, where the selection of analytes (parent compound vs metabolites) must be carefully scientifically justified and comply with regulatory requirements [57].

7.2 LC-MS/MS as the Gold Standard Bioanalytical Platform

The ultimate gold standard bioanalytical technique for the quantitative measurement of drugs and metabolites in biological matrices for BE studies is liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The high sensitivity of LC-MS/MS, which can be as low as picogram per milliliter (pg/mL), and the high selectivity, related to the mass and fragment, makes it ideal for the determination of drug levels within the extensive dynamic range of clinical PK studies. Multiple reaction monitoring (MRM) acquisition mode, which selectively monitors a specific precursor-to-product ion transition for each compound, gives the sensitivity and specificity needed to accurately quantify analytes even from complex biological matrices like human plasma. Compared to traditional analytical methods like high-performance liquid chromatography with ultraviolet detection (HPLC-UV), or gas chromatography, LC-MS/MS can provide a degree of quantitative accuracy that cannot be matched with stable-isotope-labeled internal standards that are identical to the analyte in all chemical aspects except detectable mass [58].

7.3 Regulatory Requirements for Bioanalytical Method Validation

The ICH M10 guideline on Bioanalytical Method Validation and Study Sample Analysis, adopted by ICH in May 2022 and adopted by major regulatory bodies like the USFDA, EMA and Health Canada is the latest guideline for validation requirements for bioanalytical methods for BE studies. ICH M10 lists validation parameters to be evaluated for all chromatographic assay procedures, such as selectivity, matrix effect and recovery, calibration model, accuracy and precision (within Run and between Run), dilution integrity, stability (In Processed Samples, In Biological Matrix at various storage temperature, Freeze Thaw and Stock Solutions) and carryover. The guideline includes specific acceptance criteria for each validation parameter, additional requirements for incurred sample reanalysis (ISR) as an important criterion for method reliability, and special considerations for biomarker assays and large molecule quantification methods. Conformance to ICH M10 is now a critical pre-requisite to the acceptance of BE study data by regulatory authorities in all ICH member regions while ICH harmonized requirements have significantly eliminated the discordance among the regional bioanalytical validation requirements in the past [59].

VIII. Challenges in Bioequivalence Studies

8.1 Highly Variable Drugs

Highly variable drugs are one of the most intractable and challenging problems for BE science, as they are explained in Section 6.1. In addition to the statistical power requirements, which would result in scaled acceptance criteria or larger sample sizes, the investigation of HVDs is also complicated by the challenge of separating formulation differences from the statistical "noise" of high kinetic variability. Crossover designs, where the subject receives the reference product twice (partial replicate, 3-period design) or twice receives both the test and reference product (full replicate, 4-period design) are mandatory for the RSABE and scaled average BE approaches and require more study subject compliance, washout period length, and clinical monitoring resources. In addition, the extent of regulatory acceptance and implementation of scaled BE approaches is different in different jurisdictions, meaning that a generic product that successfully fulfills the BE requirements of one regulatory authority may also need a study with different design and power to meet requirements of another regulatory authority [60].

8.2 Endogenous Compounds

Bioanalytical and pharmacokinetic challenges do exist for BE studies of drugs that are endogenous substances or their metabolites are endogenous. When the analyte of interest is normally present at measurable basal concentrations in human plasma as is the case for hormones such as estradiol and progesterone, vitamins such as vitamin D and folic acid, and endogenous mediators such as prostaglandins the baseline concentration must be carefully characterized and appropriately subtracted or accounted for in the pharmacokinetic analysis to isolate the drug-derived exposure. Different regulatory guidance exists for tackling this challenge: baseline subtraction, baseline correction via the use of pre-dose concentrations, and pharmacodynamic endpoints as alternative measures of BE. The intra-individual and diurnal variations of the endogenous baseline concentrations provide an added factor of complexity in the design and interpretation of BE studies for endogenous drugs, which may need to be more complex or higher sample size to ensure sufficient statistical power [61].

8.3 First-Pass Metabolism and Food Effect

Drugs with high first pass metabolism with large inter- and intra-individual variability in the intestinal wall or liver and whose systemic exposure is sensitive to rate of absorption/dissolution can be liable to large inter- and intra-individual differences in BE assessment, where minor differences in formulation can result in large differences in systemic exposure if the metabolic enzymes are saturated or induced. For such drugs, the correlation between in vitro dissolution and in vivo absorption is non-linear and this means that biowaiver prediction and IVIVC development is unreliable. The food effect is especially a complicated element for drugs that are highly extracted, because of the increased blood flow to the liver and intestine after food intake, which can significantly change the firstpass extraction ratio and, in turn, the systemic bioavailability of high-extraction drugs. This challenge is exacerbated by the relationship between the composition of the food, especially fat content, caloric density, and meal volume and the performance of the drug product, with high fat meals universally known to cause the largest food effect on drug product absorption, and thus being the standard food effect test condition in regulatory guidance [62].

8.4 Ethnic and Genetic Variability

Interethnic differences in pharmacokinetics are a clinically relevant source of intersubject variation in BE studies and are important with respect to global acceptance of BE data from one population for regulatory submissions for another population. The allelic frequency distributions of genetic polymorphisms of drug

metabolizing enzymes, such as cytochrome P450 enzymes CYP2D6, CYP2C19, CYP2C9 and CYP3A4/5, vary significantly between populations, so that different populations have different frequencies of individuals with varying capabilities for drug metabolism, which affects the drug metabolism profile. In the same way, genetic polymorphisms in drug transporters like P-glycoprotein (ABCB1), BCRP/ABCG2 and OATPs also account for some ethnic differences in drug absorption and disposition. Ethnic differences in pharmacokinetics have been specifically acknowledged by regulatory authorities, such as PMDA, which state that BE studies should either be performed with Japanese subjects or the lack of difference in pharmacokinetics between Japanese and non-Japanese subjects should be demonstrated in bridging studies prior to regulatory submission to ethnically different populations [63].

8.5 Regulatory Harmonization Challenges

International regulatory collaboration efforts, like the IPRP BEWGG or the ICH M13A guideline development process, have resulted in some great progress; however, there is still a significant heterogeneity in the world of BE. Variances in definitions of reference products, acceptability of foreign BE study data, requirements for local pivotal studies, handling requirements for highly variable drugs and NTI compounds, scope of biowaiver eligibility and specific bioanalytical validation standards applied result in a disjointed international landscape leading to substantial duplication of effort and cost for pharmaceutical manufacturers seeking multi-jurisdictional generic drug approvals. The survey of BEWGG member agencies clearly shows the basic preferences of all participating regulators for single-dose crossover designs in healthy subjects, but also reveals a number of differences in preference with respect to the fed versus fasted study requirement determination, the approach to multiple-dose studies, and the specific approach to endogenous compound BE assessment. While each of these differences may have scientific reasons, these differences are a hurdle to the efficiency of the regulatory process and to accessibility of affordable medicines on a global scale [64].

8.6 Cost and Logistical Challenges in Developing Countries

There are significant practical challenges for pharmaceutical manufacturers in developing countries in designing and conducting BE studies that conform to internationally accepted standards. To set up and qualify the clinical pharmacology units for BE studies under GCP conditions, a significant amount of capital investment is needed in infrastructure, clinical and monitoring personnel, and validation of bioanalytical laboratories. The analytical core of modern bioanalytical research, LC-MS/MS instrumentation, is costly to purchase and operate, and required technical expertise for operating and developing the instrument may not be available in the low and middle income country environment. In many developing countries, the regulatory capacity building is still on-going, and the lack of a complete set of BE guidelines in the respective countries means that the manufacturers need to work with several international guidelines without the support of national guideline. Together they lead to slow approval times for generic drugs, the lack of price pressure on originator products and, ultimately, an impediment to delivering low-cost medicines to the patients who need them most in certain parts of the world [65].

IX. Recent Advances and Future Perspectives

9.1 In Vitro-In Vivo Correlation (IVIVC)

In vitro-in vivo correlation (IVIVC) represents a mathematical model that describes the relationship between an in vitro property of a dosage form most commonly the dissolution or drug release rate and an in vivo pharmacokinetic response such as the plasma drug concentration-time profile or the fraction of drug absorbed. A strong and valid IVIVC, especially a Level A correlation which explains the in vitro dissolution

and in vivo absorption on a point-by-point basis, is the most scientifically meaningful and regulator accepted IVIVC and is useful in BE assessment and formulation development. Level A IVIVC could therefore be used as a tool to waive in vivo BE studies for some strength variations or manufacturing changes of a drug product, and thus is a valuable tool for reducing the clinical study burden associated with drug product lifecycle management. IVIVC is most feasible for extended release dosage forms, where dissolution is rate limiting for absorption, and where the dissolution profile can be made to mimic the in vivo absorption profile. The scientific quality and predictive power of recently developed IVIVC models have all been improved by advances in biorelevant dissolution media formulations, physiologically relevant dissolution apparatus designs, and mechanistic modeling of gastrointestinal drug absorption [66].

9.2 Physiologically Based Pharmacokinetic (PBPK) Modeling

Physiologically based pharmacokinetic modeling has become one of the most powerful advances in methodology for BE science and provides a mechanistic framework to predict and explain the pharmacokinetic behavior of drugs under a variety of physiological conditions, patient populations and formulation settings. PBPK models are used to make predictions of drug concentration-time profiles in plasma and tissues by combining mathematical equations describing drug-specific physicochemical and biochemical properties (such as solubility, permeability, protein binding, metabolic clearance, and interaction with transporters) with physiological parameters that describe the anatomy, blood flow, tissue volumes, and biochemical environment of the human body. For BE and generic drug development, PBPK modeling has been used to help predict the consequences of formulation change on systemic exposure for BE waiver, to evaluate the food effect risk for a particular drug product, to extrapolate BE results from one population to another (e.g. from adults to children) and to evaluate potential for drug-drug interactions that might be a concern for BE studies. The acceptance of PBPK modeling to support regulatory decisions around BE has increased significantly from 2020 to 2024, with the USFDA reporting 26.5% of approved new drugs had included PBPK models as pivotal evidence in regulatory submissions, demonstrating the increasing use of mechanistic modeling in the drug approval process [67].

9.3 Adaptive BE Study Designs

Adaptive study designs have created a new paradigm of statistical efficiency and scientific flexibility in the conduct of BE studies, which allows pre-planned changes to study parameters based on the data being collected in the study itself to be made without adversely impacting upon the statistical integrity of the final analysis. Most widely used adaptive designs in BE studies are those that enable for interim analyses to either evaluate for early success or futility or to reassess the sample size based on the withinsubject variability observed. The regulatory acceptance of the adaptive BE designs has grown significantly and these designs have been approved by the USFDA, EMA, Health Canada and WHO with well defined pre-specification and analysis requirements. They are especially useful when little or no information is available on the within-subject variance of a new drug entity, so that the study could be carried out efficiently without an investigation being under-powered or over-powered by over- or under-estimating the variance [68].

9.4 Role of Artificial Intelligence and Machine Learning in BE Prediction

Over the last few years, artificial intelligence (AI) and machine learning (ML) technologies have been gaining traction as tools to help improve the BE assessment process, whether it be to reduce the risk of BE issues during formulation development, to enhance the design of clinical studies, or to optimize the processing of bioanalytical data. Machine learning (ML) models developed from large databases of drug physical-chemical and generic formulation properties and in vivo PK results have shown the potential to

predict BE failure of generic formulations before they enter the clinic, which could be useful to make more intelligent choices of formulation optimization at the beginning of the development process. In the case of PBPK modelling, AI and ML are actively being studied to overcome one of the main drawbacks of mechanistic modelling – the accurate estimation of uncertain or unknown model parameters, using approaches for ML-based parameter estimation that can be adapted to large databases of physicochemical and pharmacokinetic data to extract the relevant information. The FDA has even clearly identified how AI and ML could be benefiting the industry in drug development and regulatory review, and has encouraged the pharmaceutical industry to investigate these tools and establish necessary frameworks for their regulatory validation and use. AI-powered predictive technologies, when combined with traditional regulatory BE approaches, offer a realm of significant scientific promise, with the promise of significantly shortening timelines for generic drug development and minimizing the need for resource-intensive in vivo clinical trials [69].

X. CONCLUSION

Bioequivalence assessment is still a fundamental pillar of generic drug approval, as this is the scientific pathway that links pharmaceutical equivalence with therapeutic interchangeability. In this review, we have explored the diverse aspects of BE study design, international regulatory environment, and the challenges faced by the regulators in major jurisdictions such as USFDA, EMA, WHO, CDSCO, Health Canada and PMDA. There is a general agreement on basic concepts like two period crossover design, 90% confidence interval approach and with respect to 80.00–125.00% acceptance criteria, but there are some major differences on how to deal with highly variable drugs, drugs with narrow therapeutic index, BCS-based biowaiver and foreign study data. All these inconsistencies create heavy scientific, economical, and logistical burden to generic manufacturers, especially in a developing economy. The introduction of ICH M10 and the ongoing work on ICH M13A are significant steps towards harmonisation across the world, and could significantly reduce duplication of regulation and provide access to affordable medicines globally. Newer techniques such as PBPK modelling, IVIVC, adaptive study designs and AI-based predictive tools are increasingly changing the BE landscape, providing effective alternatives or complements to conventional in vivo studies. In the future, as the science of regulation continues to develop, a collaborative science-based process between international regulatory authorities, industry and academic investigators will be required to make sure BE frameworks continue to be robust, equitable and responsive to the increasing complexity of the modern pharmaceutical product, and ultimately serve the end user of timely access to safe and effective generic medicines through the eyes of the patient.

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